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Malonyl-CoA Decarboxylase (MCD) as a Potential Therapeutic Target for Breast Cancer

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Abstract: Fatty acid synthase (FAS) inhibition initiates selective apoptosis of cancer cells both *in vivo* and *in vitro,* which may involve malonyl-CoA metabolism. These findings led to exploration of malonyl-CoA decarboxylase (MCD) as a potential novel target for cancer treatment. MCD regulates the levels of cellular malonyl-CoA through the decarboxylation of malonyl-CoA to acetyl-CoA. Malonyl-CoA is both a substrate for FAS and an inhibitor of fatty acid oxidation acting as a metabolic switch between anabolic fatty acid synthesis and catabolic fatty acid oxidation. We now report that treatment of human breast cancer (MCF7) cells with MCD small interference RNA (siRNA) reduces MCD expression and activity, reduces ATP levels, and is cytotoxic to MCF7 cells, but not to human fibroblasts. In addition, we synthesized a small molecule inhibitor of MCD, 5-{(Morpholine-4-carbonyl)-[4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl-ethyl)-phenyl]-amino}-pentanoic acid methyl ester (MPA). Similar to MCD siRNA, MPA inhibits MCD activity in MCF7 cells, increases cellular malonyl-CoA levels and is cytotoxic to a number of human breast cancer cell lines *in vitro*. Taken together, these data indicate that MCD-induced cytotoxicity is likely mediated through malonyl-CoA metabolism. These findings support the hypothesis that MCD is a potential therapeutic target for cancer therapy.

14. SUBJECT TERMS

Malonyl-CoA Decarboxylase, malonyl-CoA, siRNA

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INTRODUCTION

Our studies of cancer cell metabolism led to the observation that transformed cells exhibit high levels of fatty acid synthase (FAS) expression and fatty acid synthesis (Kuhajda, 2006; Kuhajda et al., 1994). Cancer cells are dependent upon active lipogenesis, as FAS inhibition either through siRNA or pharmacological agents, leads to cancer cell death both *in* vitro and *in vivo* (Kuhajda, 2006; Swinnen et al., 2006). Following FAS inhibition, increased levels of malonyl-CoA, the substrate contributing most of the carbon for fatty acid synthesis (Wakil, 1989), have also been hypothesized as a possible trigger for cytotoxicity (Pizer et al., 2000; Zhou et al., 2003). As such, in lipogenic cells, malonyl-CoA levels are highly regulated by synthesis, utilization as substrate, and metabolism (Figure 1). Acetyl-CoA carboxylase (ACC), the rate limiting enzyme of fatty acid synthesis, produces malonyl-CoA from the ATP dependent carboxylation of acetyl CoA (Witters et al., 1994; Witters & Kemp, 1992). This step is highly regulated by a feed-forward allosteric activation by citrate, and inhibition by both AMPK activated kinase (AMPK) and long-chain acyl-CoA's. Malonyl-CoA is the substrate which provides the predominant carbon source for the synthesis of fatty acids by fatty acid synthase (FAS). Malonyl-CoA decarboxylase (MCD) (E.C. 4.1.1.9) acts to regulate malonyl-CoA levels through its decarboxylation back to acetyl-CoA (Fig. 1) (Goodwin & Taegtmeyer, 1999). Inhibition of MCD affords another strategy to rapidly increase malonyl-CoA levels through decreasing its catabolism. Using both siRNA and pharmacological inhibition of MCD, we report that MCD inhibition increases malonyl-CoA levels in cancer cells, induces cytotoxicity, and potentiates pharmacological FAS inhibition. These findings identify MCD as a potential target for cancer therapy development.

BODY

MCD siRNA Reduces MCD Expression and Activity. To inhibit the expression and activity of MCD, we first utilized siRNA targeted to the human MCD sequence. MCD siRNA reduced both the expression and activity of MCD in MCF7 cells. As measured by quantitative RT-PCR, there was a significant reduction of MCD mRNA (Figure 1A) within 6 h, persisting through 48 h. Both protein expression by immunoblot (Figure 1B) and MCD activity (Figure 1B) were substantially reduced within 6 h following MCD siRNA transfection. Since MCD siRNA rapidly reduced both MCD expression and activity in MCF7 cells, it was deemed a useful reagent to test the effects of MCD inhibition in cancer cells.

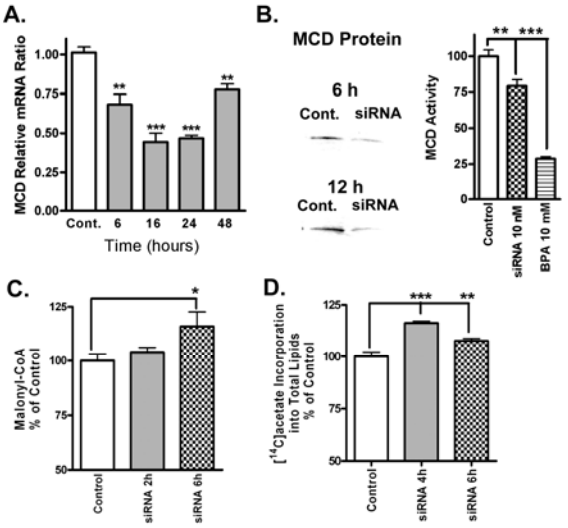

Figure 1. MCD siRNA Reduces MCD Expression and Activity.

As a consequence of MCD inhibition in MCF7 cells, malonyl-CoA levels were increased by approximately 120% 6 h following siRNA treatment (Figure 1C). Since MCF7 cells are known to express FAS and undergo lipogenesis, the increased malonyl-CoA levels also increased fatty acid synthesis as measured by [^{14}C]-acetate incorporation into total lipids. Thus, MCD inhibition led to an increase in steady-state levels of malonyl-CoA, thereby enhancing fatty acid synthesis (Figure 1D). Since FAS is not a regulated step of the pathway, it is not surprising that increased substrate availability at the FAS step led to increased flux through the pathway.

MCD siRNA is cytotoxic to MCF7 cells but not MRC-5 fibroblasts. MCD inhibition with siRNA led to rapid and selective cytotoxicity against MCF7 cells. Treated MCF7 cells exhibited morphological features of cell injury (Figure 2A, upper right panel) characterized by pyknotic and fragmented cells, while controls were unremarkable (Figure 2A, upper left panel). MRC-5 human fibroblasts (Figure 2A, lower right panel) and controls (Figure 2A, lower left panel) were essentially unaffected by siRNA treatment. Scrambled siRNA controls were similar to lipofectamine controls (data not shown). As further evidence of the cytotoxicity of MCD inhibition, MCD siRNA also reduced clonogenicity of MCF7 cells within 16h (Figure 2B). Evidence of apoptosis by positive TUNEL staining occurred within 2h (Figure 2C) and persisted through 6h.

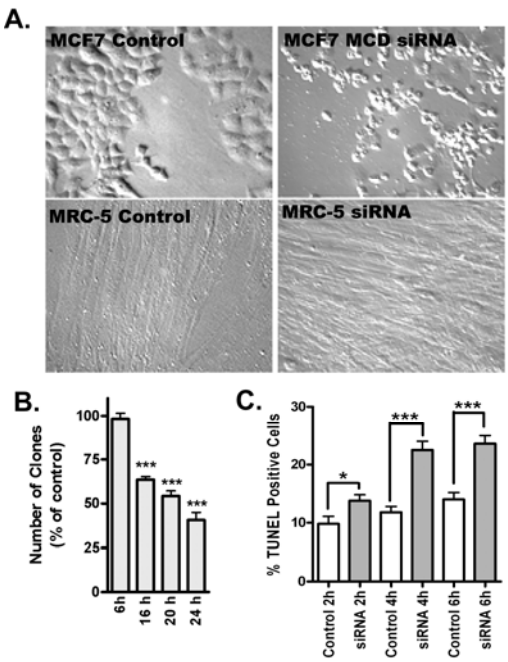

Figure 2. MCD siRNA is cytotoxic to MCF7 cells but not MRC-5 fibroblasts.

MPA, pharmacologically inhibits MCD in MCF7 cells. The structure of MPA is shown in Figure 3A. It is a small molecule MCD inhibitor reported to inhibit MCD activity in rat heart (Cheng et al., 2006). Figure 3B shows a concentration dependent inhibition of MCD activity by MPA in MCF7 cells after 2 h treatment. MPA inhibited human MCD by 30% at 80 µg/mL with 50% inhibition at approximately 160 µg/mL. Although there is a dose dependence, there is not

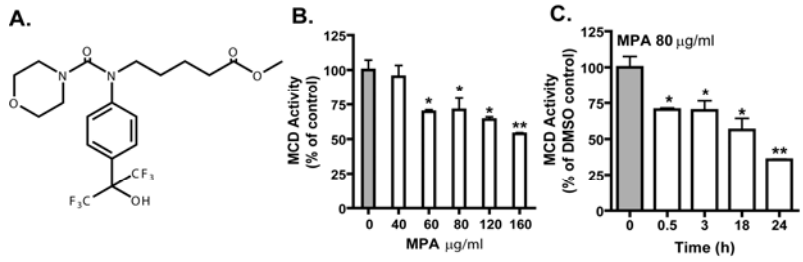

Figure 3. MPA, pharmacologically inhibits MCD in MCF7 cells

a substantial time dependence of the inhibition at 80 µg/mL as shown in Figure 3C. MPA rapidly inhibits MCD activity within 30 minutes by about 30% increasing to 65% after 24 h. The 24 h reduction is more likely due to cellular consequence of MCD inhibition rather than direct effects of MPA upon MCD.

Pharmacological inhibition of both MCD and FAS increases cellular malonyl-CoA levels while reducing free CoA. MCF7 cells were treated with MPA (80 µg/mL) or cerulenin (5 µg/mL),

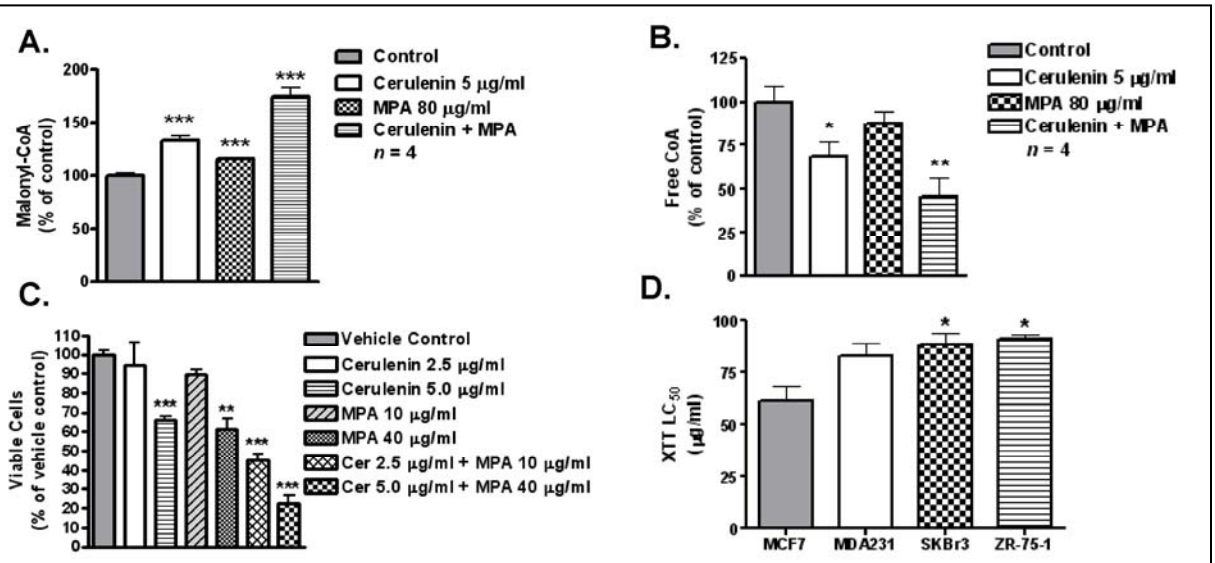

Figure 4. Pharmacological MCD inhibition increases malonyl-CoA levels, reduces free CoA, and potentiates FAS inhibition in human breast cancer cell lines.

an FAS inhibitor (Funabashi et al., 1989) for 6 h, after which malonyl-CoA levels were measured by HPLC (Figure 4A). Inhibiting FAS with cerulenin increased malonyl-CoA levels by 123% while MCD inhibition with MPA increased malonyl-CoA to 110% of control. Thus, blocking the utilization of malonyl-CoA with FAS inhibition or malonyl-CoA metabolism with MCD inhibition both increased malonyl-CoA levels. Blocking both MCD and FAS led to a 162% increase in malonyl-CoA levels. To achieve this, cells were treated with cerulenin one hour prior to the addition of MPA. Conversely, as malonyl-CoA levels increased, free CoA levels were reduced. Figure 4B

demonstrate that cerulenin reduced free CoA by 32%, MPA by 13 %, and combined cerulenin and MPA by 54%.

Acetyl-CoA levels were not substantially affected by either FAS or MCD inhibition (data not shown). Thus, both FAS and MCD inhibition increase malonyl-CoA levels seemingly at the expense of cellular free CoA reserves.

Pharmacological MCD inhibition is cytotoxic to human breast cancer cells and potentiates FAS inhibition induced cytotoxicity. MCF7 cells treated with MPA at 40 µg/mL for 24 h induced substantial cytotoxicity (Figure 4C), which was similar to cerulenin treatment at 5 µg/mL. Neither MPA at 10 µg/mL nor cerulenin at 2.5 µg/mL alone were significantly cytotoxic, however, when combined, 55% of the cells were no longer viable. The combination of MPA at 40 µg/mL and cerulenin at 5 µg/mL were more cytotoxic than either compound alone. Figure 4D illustrates the LC_{50} for MPA against a panel of human breast cancer cell lines which include estrogen receptor (ER) positive / HER2 unamplified MCF7, ER positive / HER2 amplified ZR-75-1, ER negative / HER2 amplified SKBr3, and ER negative / HER2 unamplified MDA231 (Menendez et al., 2004; Pegram et al., 2004; Shiu et al., 2008).

KEY RESEARCH ACCOMPLISHMENTS

[1] Reduction of MCD activity by both siRNA reducing its expression, or by pharmacological inhibition was selectively cytotoxic to human breast cancer cells.

[2] Exploration of the mechanism of action of MPA, the pharmacological FAS inhibitor, showed that it both inhibited MCD activity, and increased malonyl-CoA levels in cells. This demonstrates that the compound inhibits MCD *in vitro* in human cancer cells.

[3] Pharmacological MCD inhibition potentiated pharmacological FAS inhibition consistent with malonyl-CoA involvement in its mechanism of action.

[4] Pharmacological MCD inhibition reduced free CoA levels in cells which may also contribute to its mechanism of action.

With the exception of xenograft studies which are in the planning stages, all of the specific aims of the grant were achieved.

REPORTABLE OUTCOMES

[1] Presented these data at the Era of Hope meeting sponsored by the DOD in Baltimore, MD in 2007.

[2] A manuscript containing these data is under review at the journal Oncogene.

CONCLUSIONS

[1] MCD is a potential therapeutic target for breast cancer therapy.

[2] Increased cellular levels of malonyl-CoA is likely a key mediator of both MCD and FAS inhibition.

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APPENDIX

Zhou W, Tu Y, Simpson J, Kuhajda FP. Malonyl-CoA Decarboxylase (MCD) Inhibition is selectively cytotoxic to human breast cancer cells. In review at Oncogene.